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Significance of DNA Adduct Studies in Animal Models for Cancer Molecular Dosimetry and Risk Assessment

by Frederick A. Beland[1] and Miriam C. Poirier[2]

To elucidate the relationship between DNA adduct formation and tumorigenesis, a number of experiments have been conducted to measure DNA adducts in target tissues from experimental animals during continuous exposure to carcinogens. With aflatoxins, aromatic amines, and polycyclic aromatic hydrocarbons, tumor induction appears to be associated with the major DNA adduct detected, whereas with *N*-nitrosamines the response is normally correlated with minor forms of DNA damage. During continuous carcinogen administration, steady-state adduct concentrations are generally obtained in the target tissues, and there is often a linear correlation between the carcinogen concentration and the steady-state DNA adduct level. Exceptions exist when the mechanism of activation changes or with the onset of significant toxicity. Steady-state DNA adduct levels are often linearly related to the tumorigenic response. Carcinogen-induced cell proliferation occurs when significant deviations from linearity are observed. Because DNA adducts detected in humans are chemically identical to those found in experimental animals, DNA adduct data in animals may contribute to our understanding of human cancer risk.

Introduction

Recently published reports have described the presence of DNA adducts in individuals exposed to chemical carcinogens [reviewed in Poirier and Weston (*1*)]. The majority of these carcinogen exposures appear to be chronic in nature, and the DNA adduct concentrations presumably reflect steady-state levels that are the product of concomitant adduct formation and removal. In spite of this achievement, the correlation between the measured DNA adduct levels and human cancer risk is not known and may be exceedingly complex. To help elucidate this relationship, a number of investigations have been conducted using animal models in which DNA adducts were measured during the chronic administration of tumorigenic dosing regimens. In this review, we discuss these studies, emphasizing four classes of chemical carcinogens for which there is substantial evidence of human exposure: *N*-nitrosamines, aflatoxins, aromatic amines, and polycyclic aromatic hydrocarbons. In addition, we will compare the DNA adducts that have been detected during these tumorigenic dosing studies with those identified in humans.

N-Nitrosamines

Humans are exposed to *N*-nitrosamines from a wide variety of sources including foods, beverages, tobacco, cosmetics, cutting oils, hydraulic fluids, and rubber products (*2*). As with the majority of chemical carcinogens, *N*-nitrosamines tend to be chemically inert compounds that must be metabolized to reactive electrophiles before binding to cellular macromolecules (*3*). Although N7 guanine adducts or phosphotriesters typically are formed to the greatest extent, a comparison of the distribution of adducts from direct-acting alkylating agents with their tumorigenic potential indicates that alkylation of exocyclic oxygens (e.g., O^6 of guanine) is more important for the induction of tumors than reaction with the ring nitrogens [e.g., N7 of guanine (*4,5*)].

The relationship between dose and the concentration of DNA adducts during continuous carcinogen administration has been investigated by Boucheron et al. (*6*), who treated rats with various concentrations of *N*-nitrosodiethylamine for up to 70 days. In the target organ, the liver, the concentration of O^4-ethylthymidine increased rapidly at each dose for about the first 14 days, whereupon steady-state adduct levels were obtained (Fig. 1*A*). At steady state (e.g., after 49 days of continuous treatment; Fig. 1*B*), the levels of O^4-ethylthymidine were dependent on dose, with essentially a linear correlation being observed between dose and adduct concentration up to 40 ppm. At 100 ppm *N*-nitrosodiethylamine, the steady-state levels of O^4-ethylthymidine were similar to those at 40 ppm (Fig. 1*B*). This apparent failure to form more adducts may be due to increased cell turnover (*7*).

A similar study was conducted by Belinsky et al. (*8*), who measured O^6-methylguanine in DNA from different cell types in the lungs of rats treated subcutaneously three times per week for 4 weeks with five different doses of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The concentration of

[1]National Center for Toxicological Research, Jefferson, AR 72079.
[2]National Cancer Institute, Bethesda, MD 20892.
Address reprint requests to F. A. Beland, HFT-110, National Center for Toxicological Research, Jefferson, AR 72079.

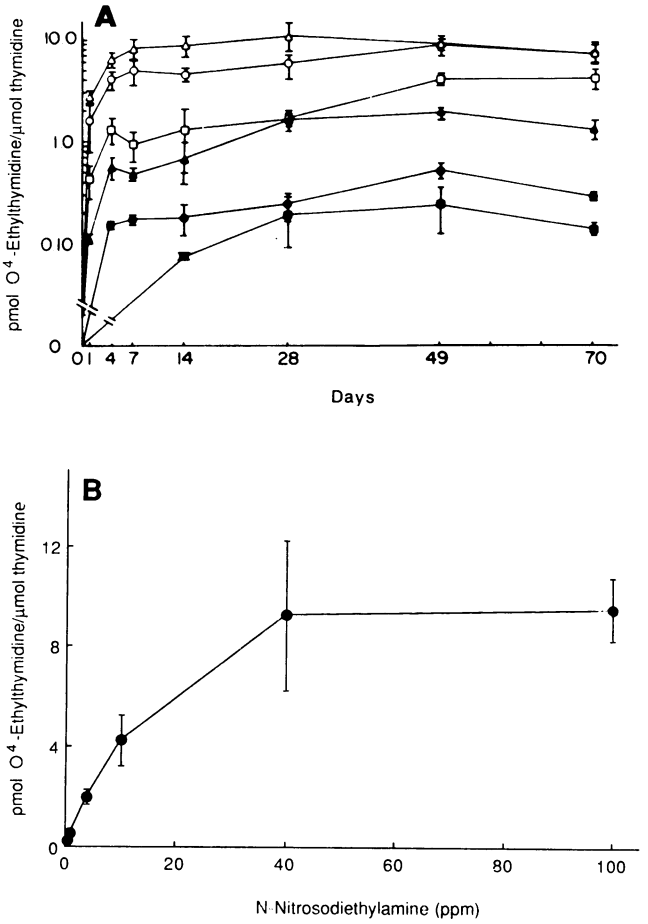

FIGURE 1. Accumulation of O^4-ethylthymidine in hepatic DNA during continuous exposure of rats as a function of (*A*) time during exposure to 0.4 (■), 1 (●), 4 (▲), 10 (□), 40 (○), or 100 (△) ppm *N*-nitrosodiethylamine for up to 70 days or (*B*) dose after exposure from 0.4 to 100 ppm *N*-nitrosodiethylamine for 49 days. Data are from Boucheron et al. (*6*).

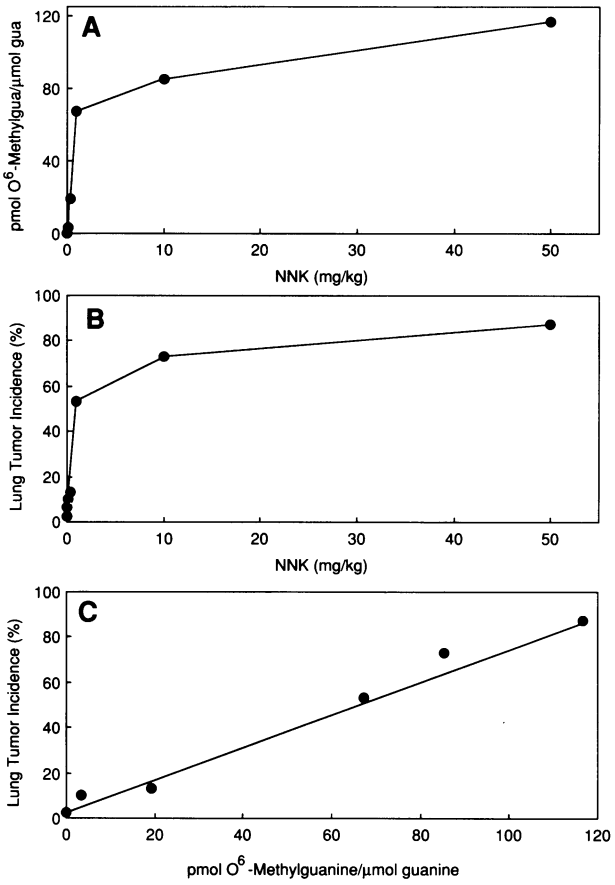

FIGURE 2. Relationship between (*A*) administered dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and concentration of O^6-methylguanine in Clara cells of rats after dosing three times per week for 4 weeks; (*B*) administered dose of NNK and lung tumor incidence after 2 years following dosing three times per week for 20 weeks; and (*C*) concentration of O^6-methylguanine in Clara cells of rats after dosing three times per week for 4 weeks and lung tumor incidence after 2 years following dosing three times per week for 20 weeks. Data are from Belinsky et al. (*8*).

O^6-methylguanine in Clara cells exhibited nonlinear kinetics with respect to dose, with adduct formation occurring to a much greater extent at lower doses than would have been expected from extrapolation of higher doses (Fig. 2*A*). This nonlinearity in response was attributed to the existence of a high-affinity pathway for the activation of NNK at low concentrations of the carcinogen. Since the kinetics of tumor induction (Fig. 2*B*) were similar to those of the adduct concentration in Clara cells (Fig. 2*A*), there was a linear correlation between the level of O^6-methylguanine in these cells and the tumor incidence (Fig. 2*C*). In the liver and nasal passages, where NNK caused tumors only at high doses, adduct and tumor profiles did not correspond. This suggests that in these tissues both adduct formation and cytotoxicity are necessary for tumorigenesis.

In another study, in which rats were treated with four IP doses of NNK, a linear relationship was observed between hepatic concentrations of N7-methylguanine and amount of NNK given (*9*). This was not the situation in the lung, which, as in the Belinsky et al. study (*8*), was attributed to the existence of a high-affinity activation pathway in lung tissue at low doses of NNK.

With the exception of the induction of oral cancer in snuff dippers (*10,11*), there is no conclusive epidemiological evidence for the carcinogenicity of *N*-nitrosamines in humans. Nevertheless, the types of DNA adducts formed in exposed individuals are similar to those observed in experimental animals. For example, Herron and Shank (*12*) found N7- and O^6-methylguanine in the liver of a victim poisoned with *N*-nitrosodimethylamine. More recently, Umberhauer et al. (*13*) detected O^6-methylguanine in stomach and esophageal tissue and esophageal tumor DNA from Chinese cancer patients. This adduct was also found at lower levels in the same tissues in Europeans, who are at a lower risk for developing esophageal tumors. Similar results were obtained by Saffhill et al. (*14*) when comparing individuals in Southeast Asia with those from England. Likewise, Huh et al. (*15*) found higher levels of O^4-ethylthymine in DNA from Japanese liver cancer patients compared to nontumor-bearing control patients. O^6-Methylguanine and O^6-ethylguanine have also been detected in peripheral lung DNA of smokers and nonsmokers, but the concentrations were not dependent on an individual's smoking status (*16*).

Aflatoxins

Humans are exposed to aflatoxins through the consumption of moldy cereals, grains, and nuts (*17,18*). Four major naturally occurring aflatoxins, aflatoxin B_1 (AFB_1), aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2, have been characterized, with AFB_1 being the most abundant as well as the most carcinogenic. The metabolic activation of AFB_1 involves oxidation of the 8,9-olefinic bond to give AFB_1-8,9-oxide (*19*), which reacts with DNA to yield *trans*-8,9-dihydro-8-(deoxyguanosin-7-yl)-9-hydroxy AFB_1 [AFB_1-N7-dG (*20*)]. AFB_1-N7-dG carries a positive charge and is therefore unstable. It can undergo depurination to give an apurinic site within the DNA (*20*) or base-catalyzed opening of the imidazole ring to yield pyrimidine adducts (*21*).

In experimental animals, AFB_1 is normally hepatocarcinogenic with the relative order of sensitivity being: trout > rat >> hamster ≅ mouse ≅ salmon (*17,22*). During the continuous administration of AFB_1, steady-state hepatic DNA adduct levels are obtained in approximately 2–6 weeks in rats (*23,24*) and 3 weeks in trout (*22*). In both species the steady-state adduct concentrations appear to be linearly related to the concentration of AFB_1 administered chronically (*24–26*). Moreover, if the steady-state adduct levels are compared to the hepatic tumor incidence, a nearly identical linear relationship is obtained for both species (*27*).

In humans, there is a positive correlation between the amount of AFB_1 ingested and the incidence of liver cancer (*17,18*). Furthermore, AFB_1 DNA adducts have been detected in tissues and urine from exposed humans (*28–30*). More recently, a highly significant correlation was observed between daily consumption of AFB_1 and concentration of serum adducts of AFB_1 and urinary AFB_1-N7-Gua (*31,32*), which presumably represent steady-state levels.

Aromatic Amines

Human exposure to aromatic amines and amides occurs from a number of sources including various industrial processes, cigarette smoke, and certain foods (*33*). There is also widespread exposure to nitropolycyclic aromatic hydrocarbons, which are products of incomplete combustion and are converted to aromatic amines by nitroreduction (*33*). In experimental animals, major target organs for tumor induction from these compounds include the liver, urinary bladder, mammary gland, and intestine. In these tissues, the major adducts are formed through covalent linkage of the amine or amide nitrogen to C-8 of guanine, while minor adducts arise from reactions between the carbons *ortho* to the amine or amide nitrogen and the exocyclic nitrogens and oxygens of guanine and adenine (*33*).

During the continuous administration of aromatic amine carcinogens to rodents, steady-state blood (*34,35*), tissue (*34*), hepatic DNA adduct (*36–38*), and bladder DNA adduct (*38*) concentrations are obtained after approximately one month of dosing. These steady-state levels are dose-related (*34,37,38*) (Fig. 3*A*), and a linear correlation exists between the DNA adduct concentration and the hepatic tumor incidence in rats (*37*) and female mice (*38*) (Fig. 3*B*) administered 2-acetylaminofluorene and in female mice (*39*) treated with 4-aminobiphenyl. However, in female mice fed 2-acetylaminofluorene (*38*) (Fig. 3*B*) and male mice administered 4-aminobiphenyl (*39*), the relationship between the bladder tumor yield and DNA adduct concentration is nonlinear. This nonlinearity in response appears to be associated with the induction of hyperplasia and implicates cell proliferation as an additional factor in the induction of bladder cancer in mice.

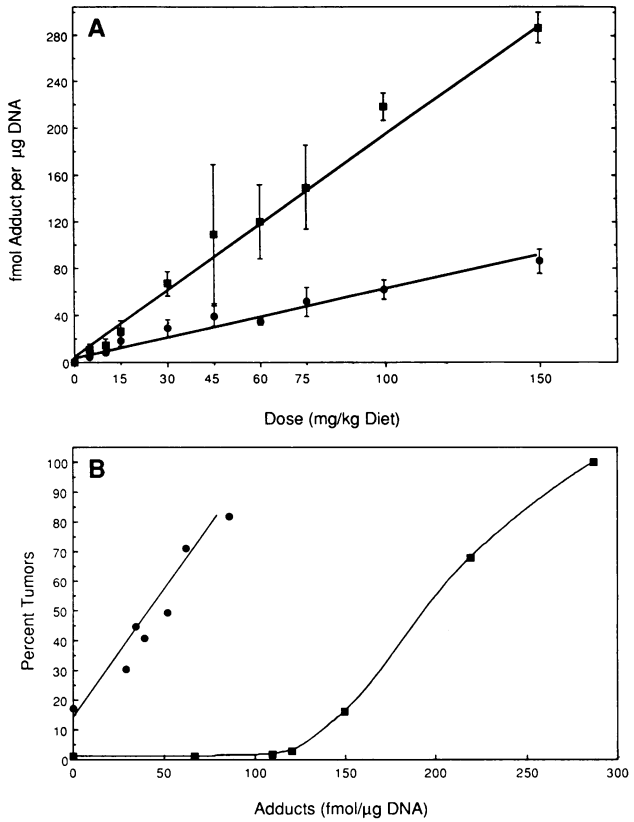

FIGURE 3. Relationship between (*A*) the dose of 2-acetylaminofluorene fed to female mice for 28 days and the concentration of *N*-(deoxyguanosin-8-yl)-2-aminofluorene in liver (●) and bladder (■) DNA and (*B*) concentration of *N*-(deoxyguanosine-8-yl)-2-aminofluorene in liver (●) and bladder (■) of female mice fed 2-acetylaminofluorene for 28 days and the tumor incidence in the same tissues after feeding the same concentrations for 33 months. Data are from Poirier et al. (*38*).

Aromatic amines are clearly associated with the induction of urinary bladder cancer in humans (*40*). Hemoglobin adducts of 4-aminobiphenyl have been detected in blood samples from humans, and these concentrations are higher in smokers as compared to nonsmokers (*41–43*), which is consistent with tobacco-related increases in the incidence of bladder cancer (*44*). In other studies, ^{32}P-postlabeling and immunoassays have indicated the presence of 4-aminobiphenyl DNA adducts in human lung and bladder samples (*16,45,46*), with the levels in the bladder being significantly higher in smokers (*46*).

Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are byproducts of combustion processes resulting in ubiquitous human exposure to this class of carcinogens (*47*). In experimental animals, PAHs induce primarily skin, stomach, lung, and mammary gland

tumors (*48*), and this appears to be the result of metabolic conversion to dihydrodiol epoxides (*49*). Generally, guanine is the preferred base for adduct formation with these reactive electrophiles; however, depending on the PAH, considerable binding can also occur with adenine and cytosine. Furthermore, because dihydrodiol epoxides are optically active (*49*), the extent of reaction with a particular nucleic acid base will depend on the particular stereoisomer being considered (*50*).

Recently, the kinetics of DNA adduct formation have been examined in the epidermis of mice administered benzo[*a*]pyrene topically once a week for 29 weeks (*51*). Steady-state adduct levels appeared to be obtained after 3 weeks of dosing, and the adduct levels were linearly related to dose up to 32 μg per treatment, whereupon a plateau occurred. As the skin tumor incidence was not linearly correlated to the dose, it was suggested that cell proliferation plays an essential role in the induction of these tumors. In preliminary studies in our laboratory, a linear relationship has been found between the adduct levels in lung and forestomach and the concentration of benzo[*a*]pyrene fed to mice for 1 month at doses up to 50 mg/kg diet (Culp and Beland, unpublished observation). The induction of forestomach tumors in mice appears to be markedly nonlinear with dose (*52*); thus, as with bladder tumors in mice (*38,39*), cell proliferation may be an additional critical factor for tumorigenesis in this tissue.

PAHs are probably carcinogenic in humans (*53*), and DNA adducts from these compounds have been detected in human populations (*1*). Inasmuch as cigarette smoke contains substantial quantities of PAHs (*54*), a number of studies have compared PAH–DNA adduct concentrations in various tissues from smokers and nonsmokers. Immunoassays with antibodies elicited against (±)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-modified DNA have revealed that smokers often have somewhat higher PAH-DNA adduct levels than nonsmokers (*55–59*), although this is not always the case (*16,60–64*). More dramatic differences have been found using ^{32}P-postlabeling, which is generally specific for "aromatic" DNA adducts. In several studies, smokers have had higher levels of discrete adducts (*55,56,65*) or a larger diffuse area of adducts (*58,66–68*) than nonsmokers; however, in only one of these studies (*58*) was a benzo[*a*]pyrene dihydrodiol epoxide-derived adduct detected. The presence of benzo[*a*]pyrene dihydrodiol epoxide DNA adducts in human placenta (*69*) and lung (*70*) has been confirmed by subjecting the DNA to immunoaffinity chromatography followed by high-pressure liquid chromatography and synchronous fluorescence spectroscopy, and also by gas chromatography–mass spectrometry in the case of the placental DNA; however, the extent of smoking did not always correlate with the adduct concentrations observed.

In another study (*71*), benzo[*a*]pyrene diol epoxide adducts were detected in alveolar macrophages by synchronous fluorescence spectrometry, and the levels did appear to depend upon smoking status. Other exposure-related increases in PAH–DNA adducts, as determined by immunoassays, have been reported in peripheral blood lymphocytes from iron foundry workers and individuals ingesting charcoal-broiled beef, and positive immunochemical results have also been found with blood samples from coke-oven workers, foundry workers, roofers, and firefighters (*1*).

Summary

In this review we have considered the DNA adducts formed in animal models from four classes of chemical carcinogens for which there is substantial evidence for human exposure. Some interesting similarities and differences between carcinogen–DNA interactions for these classes of compounds are discussed below.

The site of substitution for biologically important adducts appears to be chemical-class specific. Thus, tumor induction from *N*-nitrosamines is best associated with O^6-guanine and O^4-thymine substitution. For aflatoxins, the response is correlated with reaction at N7 of guanine; with aromatic amines, C8-guanine substitution generally appears to be the critical lesion; and for PAHs, N^2 of guanine and/or N^6 of adenine appear to be the important sites for substitution.

Tumors induced by *N*-nitrosamines are associated with the formation of minor adducts (e.g., O^6-alkylguanine and O^4-alkylthymine), whereas with the other classes of carcinogens, tumor induction is normally associated with the major forms of DNA damage.

Steady-state DNA adduct concentrations are obtained during continuous carcinogen administration, and this typically occurs after approximately 1 month of chronic dosing. Because DNA adduct levels reach steady state, an estimation of the risk for developing a tumor cannot be obtained from the DNA adduct concentration by itself, but has to include both the DNA adduct concentration and the length of carcinogen exposure.

There is often a linear correlation between the chronically administered dose levels and the steady-state DNA adduct concentrations in target tissues (e.g., 2-acetylaminofluorene in mouse liver and bladder; Fig. 3*A*). Exceptions exist when the mechanism of activation changes (e.g., NNK in rat lung; Fig. 2*A*) or with the onset of significant toxicity (e.g., *N*-nitrosodiethylamine in rat liver; Fig. 1*B*).

Steady-state DNA adduct levels are often linearly related to the tumorigenic response (e.g., 2-acetylaminofluorene in mouse liver; Fig. 3*B*). When significant deviations from linearity are observed (e.g., 2-acetylaminofluorene in mouse bladder; Fig. 3*B*), carcinogen-induced cell proliferation appears to be an additional component for tumorigenesis. Because this response occurs at relatively high carcinogen doses, it may not be germane to most human exposure scenarios.

DNA adducts can be detected in humans, and these adducts correspond to those found in experimental animals. It should be possible, therefore, to use DNA adduct data in animals to estimate human cancer risk.

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